



PATENT APPLICATION
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	: Brader, et al.)	
Serial No.	: 08/484,542 ✓)	
Filed	: 7 June 1995)	Group Art Unit:
)	1813
For	: Stabilized Acylated Insulin)	
	Formulations)	Examiner:
)	Prickril
Docket No.	: 10361.49950)	

DECLARATION UNDER 37 C.F.R. 1.131

Assistant Commissioner for Patents

Washington, D. C. 20231

Sir:

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I, Michael J. Beckage, declare that:

I am a co-inventor and an applicant in this application.

Attached exhibits 1, 2, and 3 are copies of pages from my notebook in which I recorded the preparation of a formulation comprising an aqueous solution of a fatty acid-acylated insulin and approximately 0.35 mole of zinc per mole of said fatty acid-acylated insulin, and having a pH of approximately 7.5. The fatty-acid acylated insulin was N^ε-Lys^{B29}-palmitoyl-human insulin, which is referred to as C16-insulin. The formulation also contained *m*-cresol, a phenolic compound, at a concentration of approximately 2.5 mg/mL. The dates have been redacted.

All the actions, events, and observations described in this declaration occurred in the United States, prior to 23 March 1995.

I further declare that all statements made herein of my own knowledge are true, that all statements made on information and belief are believed to be true, and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or

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imprisonment, or both (18 U.S.C. 1001), and may jeopardize the validity of the application or any patent issuing thereon.

3/10/97
Date

Michael J. Beckage
Michael J. Beckage

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Reformulation of C16-Insulin

DBF40 - UV potency 8/6/93 = 92%

Assume 90% for calculations

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U100 insulin = 3.5% mg/ml

$$U100 \text{ C16} = \frac{6046}{5808} \times 3.5\% = 1.041 \times 3.5\% = 3.6435 \text{ mg/ml}$$

$$\frac{3.6435}{0.9 \text{ mg Act/mg solid}} = 4.05 \text{ mg solids/ml}$$

dissolve in 60% final volume = 6.75 mg/ml

$$236.25 \text{ mg in } 35 \text{ ml} = 6.75 \text{ mg/ml}$$

Stock diluent

Glycerol want 16 mg/ml 26.67 mg/ml in stock

$$6.69 \text{ gm in } 250 \text{ ml } 0.01 \text{ N HCl} = 26.76 \text{ mg/ml}$$

Lot B1187 16.056 mg/ml after dilution

m-cresol density = 1.035 gms/ml

want final conc = 2.5 mg/ml

$$\text{stock conc} = \frac{2.5}{0.6} = 4.167 \text{ mg/ml} \times 35 = 145.8 \text{ mg}$$

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$$\frac{145.8 \text{ mg}}{1.035 \text{ mg/ml}} = \frac{140.86}{140.09} = 141 \lambda \text{ added}$$

B 64022 Q A 168E

Material divided in 1/2

15 ml of protein stocks to be diluted to 25 ml

Want .35 mole ratio of Zn to C16

$$3.6435 \text{ mg/ml C16 final conc} \times 25 \text{ ml} = 91 \text{ mg}$$

$$\frac{91}{6046} = .01505 \text{ m/Moles}$$

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$$\text{Want } .01505 \times .35 = 5.2679 \times 10^{-3} \text{ m/Moles}$$

$$\text{ZnO} = 81.38 \text{ mg/mMole}$$

$$5.2679 \times 81.38 = .428 \text{ mg Zn}$$

$$\frac{.428}{25} = .01712 \text{ mg/ml}$$

ZnO Stock
Y 07223

$$\frac{11}{1.4 \text{ ml } 1 \text{ M HCl}} = 7.857 \text{ mg/ml} + 1.4 \text{ ml H}_2\text{O} = 3.93 \text{ mg/ml}$$

$$\frac{.428 \text{ mg ZnO}}{3.93 \text{ mg/ml}} = .1089 \text{ ml} = 109 \lambda \text{ added}$$

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M Beckenro

Date

Verified by:

JAC

Date

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Zn containing Formulations

pink	200λ	1N	NaOH	turbid	
	25λ	1N	NaOH	turbid	
	25λ	1N	NaOH	turbid	pH 5.1
	100λ	1N	NaOH	clear	pH 7.88

QS to 25 ml divide in two parts

5λ	1N	NaOH	pH 8.05
5λ	1N	HCl	pH 8.04
10λ	1N	HCl	pH 8.0

ZA start with second 1/2 pH 7.88

grey	10λ	1N	HCl	pH 7.42	clear
	5λ	10M	NaOH	pH 7.49	

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